## MS9-O2 Time-Resolved Serial Femtosecond Crystallography On Photoswitchable Fluorescent Proteins

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Reversibly photoswitchable fluorescent proteins (RSFP) are essential tools in advanced fluorescence nanoscopy of live cells (such as PALM and RESOLFT). They can be repeatedly toggled back and forth between a fluorescent (*on*) and a non-fluorescent (*off*) state by irradiation with light at two different wavelengths. Mechanistic details of photoswitching, in particular on the ultra-fast photochemical time scale, remain largely unknown. Our consortium combines time-resolved serial femtosecond crystallography (TR-SFX) at X-ray free electron lasers, ultrafast absorption spectroscopy in solution and simulation methods to study *off*-to-*on* photoswitching intermediates in two RSFP on the picosecond to microsecond time scale. Two major bottlenecks had to be passed before TR-SFX could be conducted, *i.e.* the production of well-diffracting microcrystals in large quantities and efficient inline pre-illumination to photoswitch RSFP microcrystals from the *on* to the *off* state prior to injection. First pump-probe TR-SFX experiments were conducted at both the LCLS and SACLA that, together with time-resolved absorption spectroscopy, provide first insight into a possible sequence of events involved in photoswitching.

**Keywords:** time-resolved serial femtosecond crystallography, XFEL, microcrystals, fluorescent proteins, chromophore

## MS9-O3 Changes in metal coordination are required to regulate activity of bacterial phosphodiesterases, implicated in c-di-GMP regulated biofilm dispersal

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Many chronic infections are underpinned by the ability of bacteria to transition to the biofilm life-style, which is up to 1000 fold more tolerant to antibiotics. Both biofilm formation and dispersal are controlled by the secondary messenger bis-(3'-5') cyclic dimeric guanosine monophosphate (c-di-GMP); high levels of c-di-GMP are associated with biofilm formation, while a reduction in c-di-GMP induces biofilm dispersal[(1)]. Future pharmaceutical strategies may interfere with these mechanisms. We look at biofilm dispersal, studying c-di-GMP hydrolysis by bacterial phosphodiesterases of the EAL type.

Structures of the EAL type enzymes are readily observed in the substrate bound state (c-di-GMP). These structures pose the question of full enzyme activation; while dimerisation is known to play a key role, many of the structures are observed as dimers[(2,3)]. We present an analysis of these dimers and show that dimerisation alone is insufficient for activation.

Our study of the *Pseudomonas aeruginosa* protein MorA demonstrates that dimerisation is linked with reorganisation of the catalytic site, by unwinding of a helical segment (figure 1)[(4)]. However, this structure is again observed in the substrate bound state. We now present further evidence from phosphodiesterase structures in complex with the hydrolysis product 5'-phosphoguanylyl-(3'-5')-guanosine. Observed differences in metal coordination in the catalytic centre may represent the final layer of enzyme activation. Understanding the full catalytic potential of EAL-type phosphodiesterases is required to explore this class of enzymes in drug design.

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